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Modelling exchange kinetics of copper at the water-aquatic moss (*Fontinalis antipyretica*) interface: Influence of water cationic composition (Ca, Mg, Na and pH)

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ABSTRACT

The present study investigated the effect of water cationic composition (Ca, Mg, Na, pH) on the bioaccumulation and elimination rates of copper by an aquatic moss (*Fontinalis antipyretica*), under laboratory conditions. For this purpose, mosses were exposed to copper at an environmentally relevant and usually non-toxic concentration (5 $\mu g\,L^{-1}$) in natural waters where cationic composition and concentrations were varied.

To describe copper bioaccumulation by aquatic mosses, a two-compartment model was the first-order kinetics, was developed and calibrated under a wide range of water cationic composition. Bioaccumulation rates of Cu in mosses were significantly reduced as the concentrations of competitive cations in solution increased. Hence, in hard-water, Ca and Mg cations play a protective role as they compete with Cu^{2+} ions for the absorption on transport sites at the organism–water interface. Based on the relationships between each major cation concentration and the exchange kinetics on mosses, the binding constants (K_{C_iBL}) of each competing cations to the biological surfaces were derived. Using the present cationic-dependant kinetic model, it is now feasible to incorporate water cationic composition in the (re)interpretation of bryophytes contamination levels and in the (re)definition of Water Quality Criteria (WQC) as illustrated through two selected examples of biomonitoring programmes. In the framework of future national water quality guidelines revisions, a such flexible and mechanistic biomonitoring tool (integrating the protective effects of competing cations) may greatly improve the ability of regulators to derive site-specific Cu (metal) guidelines for protecting aquatic biota, while limiting the use of conservative assumptions.

1. Introduction

The regulatory context related to the monitoring of water systems (e.g. the European Water Framework Directive) requires that Water Quality Criteria (WQC) and surveillance networks are based on biologically relevant endpoints, e.g. concentrations of pollutants accumulated in biota. In order to provide biologically meaningful estimates of metal contamination in natural waters, biological monitors have frequently been used in various monitoring programs (Claveri et al., 1994, 1995; Croteau et al., 1998; Cenci, 2001). Among primary producers, aquatic mosses, because of (i) their widespread distribution in lotic ecosystems (if not present, mosses are easily transplanted to sites under investigation (Fernandez et al., 2006)), (ii) their ecological importance, (iii) their ability to accumulate trace metals to easily measurable levels (Claveri et al., 1994), and (iv) their ability to rapidly (days to weeks) respond to a change in ambient metal concentration (Nimis et al., 2002), fulfil most of the criteria for suitable environmental biomonitoring, as shown by their use in various monitoring programs (Cenci, 2001). Despite their long history as biomonitors, there has been little attempt to develop theoretically based models to rigorously relate metal concentrations in mosses to those in water. Presently, the measurements of trace metal concentrations in mosses exposed to polluted waters are often simply compared to background metal concentrations to classify water systems as to their relative degree of metal pollution. There is a need to put biomonitoring studies using mosses on a theoretical foundation by quantifying the influence of various environmental variables on metal accumulation by these plants.

Recently, models incorporating bioavailability of metals (and thus their potential toxicity to aquatic organisms) into operational

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risk assessments have also been proposed to assess their potential impact on biota. For example, generalizing the concept of the free ion activity model (FIAM, (Morel and Hering, 1993)), Biotic Ligand Models (BLMs) investigate how metal speciation and the competing cations (majors cations and/or pH) alter metal binding with biological sites (De Schamphelaere and Janssen, 2002; Heijerick et al., 2002; Niyogi and Wood, 2004; Borgmann et al., 2005). The experience gained on other organisms (e.g. invertebrates and fish tested in the frame of BLM development) suggests that cationic concentration in water could have a significant influence on bioaccumulation in mosses (even if the endpoint investigated here, based on bioaccumulated concentration, is different as the toxic endpoints commonly used in the BLMs). The present study aims at evaluating the competing effects of water cationic composition (Ca, Mg, Na and pH) on the exchange kinetics of copper by an aquatic moss (*Fontinalis antipyretica*). If such effects are actually verified, they could indeed be incorporated in the interpretation of bryophytes contamination levels and in the definition of associated WQC.

The methodology adopted in this study was thus based on the experimental and modelling analysis of the exchange kinetics of copper (at environmental and usually non-toxic concentrations; 5 μg L^{-1}) at water–bryophytes interface, in natural waters presenting various cationic compositions (Ca, Mg, Na, pH). The competing effects of these major cations were characterized by the affinity constants of the competing cations to the biological ligand (BL), derived from the non-linear relationships between each cation concentration and the metal uptake rate constants. Through two selected examples, we illustrate how the modelling approach described in this paper could be applied for the (re)interpretation of bryophytes measurements routinely collected in biomonitoring programmes.

2. Materials and methods

2.1. Biological material sampling

Bryophytes (*F. antipyretica*) were sampled in January and March 2006, in the Sauldre River (north-eastern France), far from significant anthropic pressure (third Strahler stream ordering). To limit biological variability, only new green tips (2–3 cm) were kept; dark green tips were discarded. The selected green tips were then placed in clean plastic bags and stored in a cool room (4 °C) prior to the experiments. Just before immersion in the exposure tanks, the tips were rewashed three times in the mineral water used for the test.

2.2. Test solutions

Exposure solutions consisted of mineral water (Mont Calm®, France, pH = 6.8, dissolved solids = 27.5 mg L^{-1}, dissolved organic carbon <0.4 mg C L^{-1}), with the following cationic characteristics: (Ca^{2+}) = 3.1 mg L^{-1}; (Mg^{2+}) = 0.64 mg L^{-1}; (Na^{+}) = 1.43 mg L^{-1}; pH = 6.8. This mineral water was spiked with different volumes of stock solutions of $CaCl_2$, $MgCl_2$, NaCl, HCl, and NaOH (Merck, Darmstadt, Germany, trace metals <0.001%), to obtain five different concentrations for each of the investigated cations: (Ca^{2+}): 3.1, 9.9, 19.7, 41.1 and 153.1 mg L^{-1}; (Mg^{2+}): 0.64, 2.1, 5.1, 10.4 and 48.8 mg L^{-1}; (Na^{+}): 1.43, 3.2, 11.0, 21.6 and 103.5 mg L^{-1}; pH: 8.4, 7.7, 6.8, 5.9 and 4.8. To investigate how cationic composition alters copper bioaccumulation, the concentration of a given cation was varied, while keeping all other cationic concentrations constant. Each exposure solution was spiked with a constant nominal dissolved copper concentration (5 μg L^{-1}). In order to limit the fixation of copper on the walls, the tanks were emptied and refilled with new solution before immersing the bryophytes.

2.3. Bioaccumulation experiments

The experimental procedure for the bioaccumulation biotests were conducted in three successive steps:

(i) Pre-equilibration step

To pre-equilibrate biotic ligand sites and cations before copper exposure, 10 g (fresh weight) of bryophytes tips were exposed for 48 h in a solution spiked with the cationic composition studied, but deprived of copper contamination.

(ii) Accumulation step

After the pre-equilibration step, bryophytes (~10 g fresh weight) were removed (0.5 mm filtered), and placed in a 15-L stirred tank in the presence of copper (batch experiments without renewal of water). The kinetics of total dissolved copper concentrations ($[Cu_W]$) was followed for 72 h according to an exponential base timing scale ($n = 15$). Afterward, aliquots of bryophytes (approx. 700 mg fresh weight per triplicate) were sampled. Extra- and intracellular copper concentrations were measured (see next section).

(iii) Desorption step

Cu exposed bryophytes were sampled at the end of the accumulation step and were resuspended (~9 g fresh weight) in a 3-L tank containing a solution with the same cationic composition as for the accumulation step, but deprived of copper contamination. The desorbed copper ($[Cu_W]$) was kinetically followed for the 24-h desorption period ($n = 6$).

2.4. Intracellular and extracellular metal content of bryophytes

At the end of the accumulation step, a triplicate of bryophytes (approximately 700 mg fresh weight) were washed for 60 min with 50 mL of 1.0 mM EDTA (Na_2-EDTA, Acros Organics, Geel, Belgium) to remove the metals adsorbed to the cell wall (Vázquez et al., 1999; Meylan et al., 2003; Fernandez et al., 2006). Copper in the EDTA solution is attributed to the extracellular metal content. Cu in the EDTA solution was measured by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS – Varian, SpectrAA-800, Varian). The washed bryophytes were dried at 60 °C for 72 h and acid-mineralized (Merck, Darmstadt, Germany, 65% suprapur nitric acid) for the measurement (by GF-AAS) of copper attributed to the intracellular metal content of bryophytes.

2.5. Water analysis

Water samples for total dissolved copper analysis were collected in triplicate, filtered with disposable PTFE syringe filters (0.2 μm, VWR International, USA), and then acidified with analytical grade HNO_3 to pH < 2 prior to storage (4 °C). Water samples for major cations (Ca, Mg and Na) were collected in triplicate for each test at the beginning of each exposure period and measured by ICP-AES.

2.6. Mass budgets

The mass budgets for copper were checked at the end of the accumulation step ($t = 72$ h) by comparing total bioaccumulated copper (in μg of Cu) and total dissolved copper lost from bulk solution (in μg of Cu).

2.7. Kinetic modelling

The key processes that control metal uptake and release by *F. antipyretica* are generally described using a two-compartment model with the first-order kinetics (Claveri et al., 1994; Croisetiere et al., 2005) (Fig. 1).

According to the model's first-order kinetics assumption, the evolution of the copper concentrations in the three different fractions (dissolved bioavailable in water Cu_W, extracellular $CuBL_1$ and intracellular $CuBL_2$ copper) are modelled as follows:

$$\frac{d[Cu_W]}{dt} = -k_1 \cdot \{BL_1\}_{free} \cdot [Cu_W] + \frac{m_{bryos}}{V} \cdot k_{-1} \cdot \{CuBL_1\} \quad (1)$$

$$\frac{d\{CuBL_1\}}{dt} = \frac{V}{m_{bryos}} \cdot k_1 \cdot \{BL_1\}_{free} \cdot [Cu_W] - k_{-1} \cdot \{CuBL_1\} - k_2 \cdot \{BL_2\}_{free} \cdot \{CuBL_1\} + k_{-2}\{CuBL_2\} \quad (2)$$

$$\frac{d\{CuBL_2\}}{dt} = k_2 \cdot \{BL_2\}_{free} \cdot \{CuBL_1\} - k_{-2} \cdot \{CuBL_2\} \quad (3)$$

where k_1 ($g mol_{BL_1}\, s^{-1}$) and k_2 ($g mol_{BL_2}\, s^{-1}$) are the adsorption and internalization rate constants. k_{-1} and k_{-2} (s^{-1}) are the rate constants for copper desorption and elimination processes; m_{bryos} is the mass (in g_{bryos} d.w.) of bryophytes suspended is the 15 l solution (V); $\{BL_1\}_{free}$ and $\{BL_2\}_{free}$ are the free extra- and intracellular site concentrations ($mol\, g^{-1}$); $[Cu_W]$, $\{CuBL_1\}$ and $\{CuBL_2\}$ are, respectively, the dissolved ($\mu g L^{-1}$), the extracellular ($\mu g g^{-1}$ d.w.) and the intracellular ($\mu g g^{-1}$ d.w.) copper concentrations. Please note that the dissolved copper in water Cu_W is there considered to be the bioavailable copper fraction since biotests were carried out in mineral water (deprived of organic ligands).

The kinetic distribution of copper in the system will thus be partly governed by the initial available free extra- and intracellular site concentrations ($\{BL_1\}_{free}$ and $\{BL_2\}_{free}$), which will decrease as the concentrations of competitive cations (such as Ca, Mg, Na, H; see Fig. 1) increases. In addition, to conform with experimental measurements and simplify Eq. (1)–(3), the conditional rate constants k'_1 and k'_2 (in s^{-1}), depending on the extra- and intracellular site concentrations $\{BL_1\}_{free}$ and $\{BL_2\}_{free}$), were defined as follows:

$$k'_1 = k_1 \times \{BL_1\}_{free} \quad (4)$$

$$k'_2 = k_2 \times \{BL_2\}_{free} \quad (5)$$

Eqs. (1)–(5) were used to derive specific and identifiable analytical solutions (details of the mathematical developments describing the changes in copper concentrations in water ($[Cu_W]$) and in the bryophytes ($\{CuBL_1\}$ and $\{CuBL_2\}$) (analytical solutions are detailed in Supplementary materials), and can be found in (Ciffroy et al., 2001)). Analytical solutions were used to fit experimental data ($[Cu_W]$, $\{CuBL_1\}$ and $\{CuBL_2\}$) by calibrating the kinetic parameters according to the procedure described hereafter.

The conditional adsorption rate constant k'_1, the desorption rate constant k_{-1} and the ratio k'_2/k_{-2} were calculated using the boundary conditions (initial conditions and steady state) of the experimental accumulation step.

Based on our previous results (Ferreira et al., 2008), we assume that at $t \sim 0$, exchanges are mainly dominated by the accumulation from water to external binding sites $\{BL_1\}$. Consequently, Eq. (1) can be simplified by deleting the desorption term ($\frac{m_{bryos}}{V} \cdot k_{-1} \cdot \{CuBL_1\}$).

Hence, the conditional adsorption rate constant k'_1 is estimated after a short exposure time (t = 5 min) as follows:

$$k'_1 = -\left.\frac{d[Cu_W]}{dt}\right|_{t=5min} \times \frac{1}{[Cu_W]_{t=0}} \quad (6)$$

when a plateau is reached for $[Cu_W]$ at t = 72 h, Eq. (1) becomes:

$$k_{-1} = k'_1 \times \frac{V}{m_{bryos}} \times \frac{[Cu_W]_{t=72h}}{\{CuBL_1\}_{t=h}} \quad (7)$$

where $[Cu_W]_{t=72h}$ and $\{CuBL_1\}_{t=72h}$ are the total dissolved copper (in $\mu g L^{-1}$) and EDTA-washed copper (in $\mu g g^{-1}$ d.w.) on the bryophytes measured at t = 72 h.

Assuming equilibrium at t = 72 h, Eq. (3) can also be simplified, assuming a null value for the kinetic term $\frac{d\{CuBL_2\}}{dt}$. The k'_2/k_{-2} ratio can thus be calculated as follows:

$$\frac{k'_2}{k_{-2}} = \frac{\{CuBL_2\}_{t=72h}}{\{CuBL_1\}_{t=72h}} \quad (8)$$

2.8. Methodology for determining constants of competing cations to the biotic ligand $K_{C_iBL_1}$

According to the general concepts of metals and cations binding to biological surfaces (Xue et al., 1988), the binding of metal and cations $[C_i]$ to the free external binding sites $\{BL_1\}_{free}$ can be characterized by a stability constant $K_{C_iBL_1}$ calculated as follows:

$$K_{C_iBL_1} = \frac{\{C_iBL_1\}}{[C_i] \times \{BL_1\}_{free}} \quad (9)$$

In addition, the maximal complexing capacity of the biotic ligand $\{CC_{BL_1}\}_{max}$ equals the sum of the concentrations of the free external binding sites $\{BL_1\}_{free}$, metal bound to the external binding sites $\{CuBL_1\}$ and competitors bound to the external binding sites $\sum_i\{C_iBL_1\}$. Under equilibrium conditions, it becomes:

$$\{CC_{BL_1}\}_{max} = \{BL_1\}_{free} + \{CuBL_1\} + \sum_i \{C_iBL_1\} \quad (10)$$

Moreover, at the low copper concentrations used for the experiment, it can be assumed that metal bound to the external binding sites is negligible compared to competitors bound to the external binding sites ($\{CuBL_1\} \ll \sum_i\{C_iBL_1\}$). Hence, combining Eq. (9) and (10), the free biotic ligand site concentration $\{BL_1\}_{free}$ can be expressed as follows:

$$\{BL_1\}_{free} = \{CC_{BL_1}\}_{max} \times \left(\frac{1}{1 + \sum_i K_{C_iBL_1} \times [C_i]}\right) \quad (11)$$

Combining Eq. (11) and (4), we obtained:

$$k'_1 = k_1 \times \{CC_{BL_1}\}_{max} \times \left(\frac{1}{1 + \sum_i K_{C_iBL_1} \times [C_i]}\right) \quad (12)$$

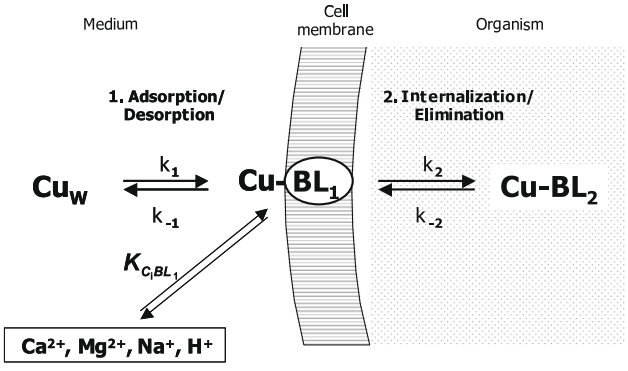

Fig. 1. Conceptual model of physicochemical processes involved in the uptake/release of Cu by aquatic bryophyte. Cu_w, $Cu–BL_1$ and $Cu–BL_2$ are the dissolved in the water, extracellular and intracellular copper, respectively.

The stability constant $K_{C_iBL_1}$ was thus hyperbolically related to k_1' and could be estimated using Eq. (12) and minimizing the quadratic error between measured and calculated k_1' values.

3. Results

3.1. Mass budgets

Copper budgets were checked at the end of the experiments (t = 72 h). There was no significant difference (ANOVA, $p < 0.05$) between the 72-h bioaccumulated fraction and the dissolved copper losses in solution. This result confirms that adsorption on tanks is negligible during the bryophytes exposure.

3.2. Kinetic accumulation of copper by bryophytes

The kinetic copper accumulation by bryophytes is indirectly shown by the depletion of copper in solution expressed by the ratio of Cu concentration at a given time to the initial Cu concentration in solution, ($[Cu_W]/[Cu_W]_{t=0}$) (Fig. 2). For each of the bioaccumulation experiments, the $[Cu_W]/[Cu_W]_{t=0}$ ratio rapidly drops and reaches a quasi plateau indicating a steady state at the water-organism interface at the end of the experiment (t = 72). Steady states at the water-organism interface are reached at different times, depending on the concentrations of the competing cations in water (increases in cationic concentrations result in delayed times to reach the steady state at the water-organism interface).

The comparisons between the measured and calculated values (analytical solutions derived from Eqs. (1)–(5) for the sorption and release experiments are shown in Fig. 2. The correlation coefficients between measured and calculated values are generally higher than 0.92, indicating that the two-compartment model properly describes the exchange kinetics of copper at the water-aquatic mosses.

For each cation biotest, the conditional metal adsorption rate k_1' determined from the experimental data of accumulation experi-

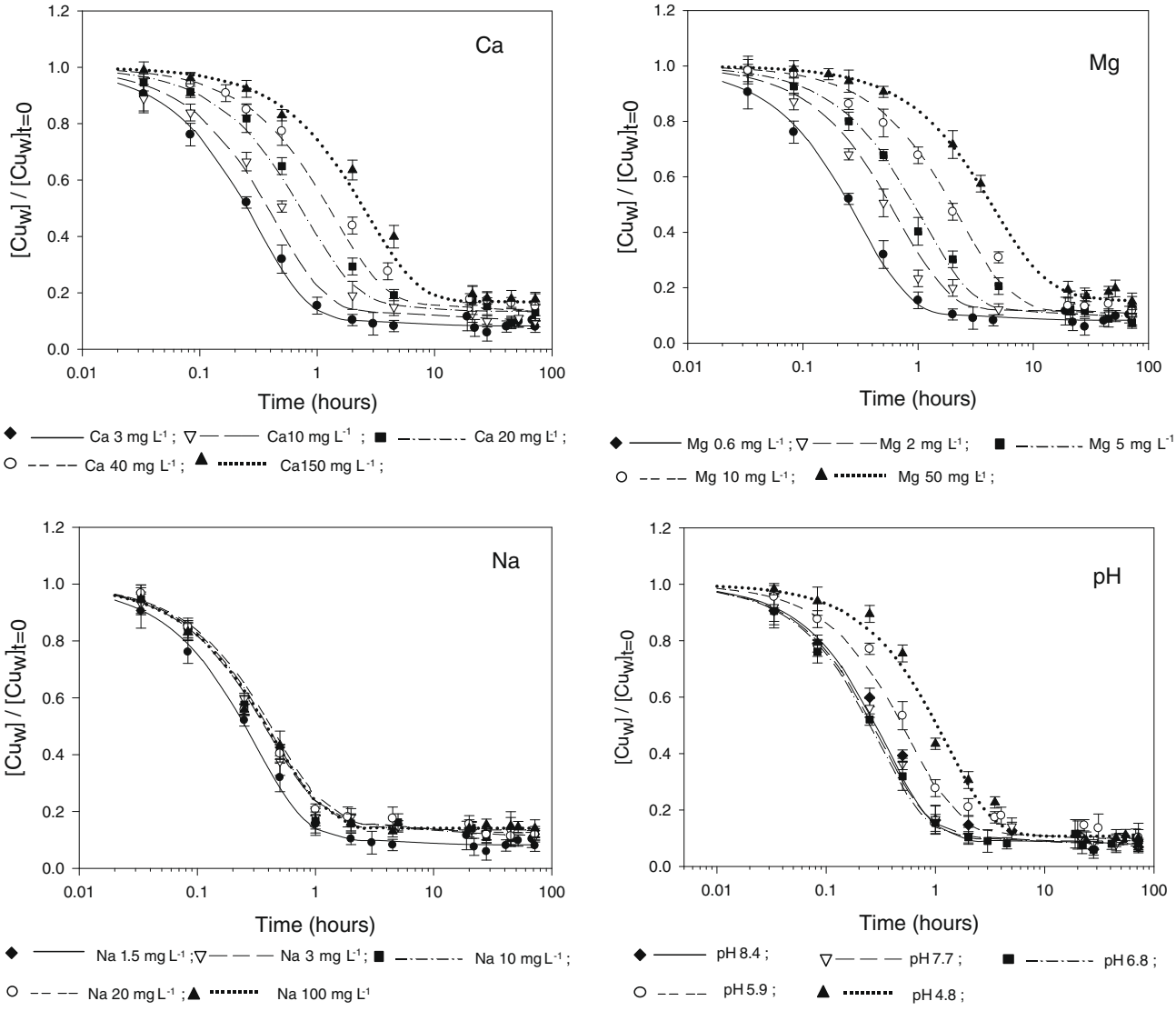

Fig. 2. Kinetic Cu loss in solution ($[Cu_W]/[Cu_W]_{t=0}$) in the various exposure media (with variable Ca, Mg, Na and pH concentrations) over 72 h of exposure. Note that the x-axis is on a logarithmic scale. Symbols and lines represent experimental and modelled data (analytical solutions derived from Eq. (1)–(5).

Table 1
Adsorption–desorption rate constants (k'_1, k'_{-1}) and internalization/elimination ratio values (k'_2/k_{-2}) as a function of the experimental conditions of each bioaccumulation biotest.

Bioassay set	Ca^{2+} ($mg L^{-1}$)	Mg^{2+} ($mg L^{-1}$)	Na^{+} ($mg L^{-1}$)	pH	[a]k'_1 (s^{-1})	[a]k_{-1} (s^{-1})	[a]k'_2/k_{-2}
Ca	**3.1**	0.64	1.42	6.85	5.33*E*−04	1.31*E*−05	0.30
	9.9	0.63	1.56	6.90	3.67*E*−04	1.02*E*−05	0.32
	19.7	0.58	1.52	6.71	2.02*E*−04	9.52*E*−06	0.28
	41.1	0.54	1.47	6.80	1.67*E*−04	1.14*E*−05	0.28
	153.1	0.60	1.44	6.69	7.78*E*−05	6.62*E*−06	0.28
Mg	3.1	**0.64**	1.39	6.91	5.33*E*−04	1.42*E*−05	0.30
	3.3	**2.1**	1.53	6.92	3.65*E*−04	1.61*E*−05	0.29
	3.1	**5.1**	1.49	6.86	2.23*E*−04	9.06*E*−06	0.28
	3.0	**10.4**	1.44	6.88	1.61*E*−04	9.62*E*−06	0.27
	2.9	**48.8**	1.41	6.75	8.29*E*−05	6.29*E*−06	0.35
Na	3.0	0.61	**1.43**	6.79	5.33*E*−04	1.42*E*−05	0.30
	3.2	0.60	**3.21**	6.78	4.33*E*−04	1.16*E*−05	0.29
	3.0	0.55	**11.0**	6.94	4.45*E*−04	1.50*E*−05	0.33
	2.9	0.51	**21.6**	6.88	4.79*E*−04	1.92*E*−05	0.28
	2.8	0.57	**103.5**	6.80	5.02*E*−04	7.80*E*−05	0.29
pH	2.8	0.62	1.46	**8.40**	6.05*E*−04	1.35*E*−05	0.28
	2.9	0.56	1.49	**7.70**	5.99*E*−04	2.08*E*−05	0.28
	3.0	0.60	1.54	**6.80**	5.33*E*−04	1.34*E*−05	0.30
	3.2	0.65	1.58	**5.90**	3.08*E*−04	1.29*E*−05	0.29
	3.1	0.66	1.44	**4.80**	1.99*E*−04	1.99*E*−05	0.29

Bold values refer to the water quality parameter varied for each bioassay set (Ca, Mg, Na, pH).
[a] k'_1, k_{-1} values and k'_2/k_{-2} ratios determined from the experimental data of accumulation experiments (Eqs. (6)–(8)).

ments (Eqs. (6)–(8)) decreased with increasing Ca^{2+}, Mg^{2+} and H^+ (but not Na^+) concentrations (Table 1). This observation supports the concept of competitive binding of these cations on transport sites at the organism–water interface.

Whatever the bioassay, the (k'_2/k_{-2}) ratio (Eq. (8)), which describes the distribution of copper (intra/extra-cellular) at equilibrium ($t = 72$ h) within aquatic mosses, remains fairly constant ($(k'_2/k_{-2})_{t=72h} = 0.294 \pm 0.02$; $n = 17$) (Table 1). This suggests that the internalization process is not influenced by cationic variations in the exposure medium.

3.3. Desorption experiments

Experimental and calculated copper concentrations during the 24-h desorption period are depicted in Fig. 3. Increases in cationic concentrations result in higher released amounts of copper from mosses in solution. This observation suggests that desorption kinetics depends on Ca^{2+}, Mg^{2+} and H^+ (but not Na^+) concentrations in solution. The values calculated for the desorption step were derived from Eq. (1)–(5), and these values are fairly well fitted with the measured copper in solution (Fig. 3), even if some gaps (mostly at low copper concentrations in solution) exist between calculated and measured values at the beginning of the experiments. This results clearly indicate that release processes are well described by the two-compartment model (see the next section below).

4. Discussion

4.1. Validation of the kinetic model

The data obtained during the desorption step (Fig. 3) are used for the validation of the two-compartment kinetic model. This dataset is compared to values which were calculated by using kinetic parameters previously calibrated from the accumulation dataset only (kinetic parameters are presented in Table 1). Thus, comparing the observed and calculated dissolved concentrations for each biotest showed that desorption experiments are well predicted, errors being lower than a factor of 2 in 90% of the cases ($n = 100$); this confirms the predictive capacity of the kinetic model developed in this study. However, for three calcium biotests, the observed copper concentrations fall below predicted concentrations, likely because of the low measured copper concentrations, which were very close to the copper detection limit (0.2 $\mu g L^{-1}$).

4.2. Effects of major cations on short-term Cu bioaccumulation kinetics

An increase of the calcium concentration from 3.1 to 153.1 $mg L^{-1}$ (0.077–3.8 mM) reduced the adsorption rate constant k'_1 with a factor 6.8 (Fig. 4a; measured k'_1 varied from 5.33×10^{-4} to 7.78×10^{-5} s^{-1}). A similar effect is observed for magnesium, i.e., a significant decrease (of a factor 6.4) in adsorption rate constant k'_1 (Fig. 4b; measured k'_1 varied from 5.33×10^{-4} to 8.29×10^{-5} s^{-1}) was observed when magnesium concentration in solution was increased from 0.64 to 48.8 $mg L^{-1}$ (0.026–2.0 mM). These findings suggest that calcium and magnesium compete with Cu for binding sites, indicating that these cations share the same uptake sites with Cu at the cell-surface ligands.

However, no significant change in the adsorption rate constant k'_1 (Fig. 4c; measured k'_1 varied from 5.33×10^{-4} to 4.33×10^{-4} s^{-1}) was found when sodium in solution varied between 1.43 and 103.5 $mg L^{-1}$ (0.062 and 4.5 mM). This result indicates that Cu uptaken by aquatic mosses is not competitively inhibited by sodium ions in solution, suggesting that sodium and copper ions do not share the same binding sites on cell membrane.

Fig. 4d shows that between pH 4.8 and 6.8, the adsorption rate constant k'_1 increased from 6.05×10^{-5} to 1.99×10^{-4} s^{-1} (factor 3.0). Increase of the pH to 8.4 do not affect significantly k'_1 (5.99×10^{-4} s^{-1} for pH 7.7 and 6.05×10^{-4} s^{-1} for pH 8.4). On the basis on these results two distinct phases can be identified (graphically represented in Fig. 4d) : (i) a first phase where increasing pH (from 4.8 to 6.8) results in a continuous increase of the number of deprotonated sites ($\{BL_1\}_{free}$) onto the cell membrane, and thus in increase of the Cu adsorption rate constants by aquatic mosses (k'_1) ; (ii) a second phase where increases in pH (from pH 7.7 to 8.4) do not result in significant increases of the deprotonated sites, and thus in the observed adsorption rate constants K'_1. These observations clearly show that copper accumulation did not depend on free copper concentration only, since the adsorption rate constant (k'_1) differed by more than a factor 6.8 when cationic composition were varied in environmentally relevant ranges.

4.3. Binding constants of competing cations to the biological surfaces

The affinity constants $K_{C_iBL_1}$ (in l mol^{-1}) and the maximal uptake rate constant ($k_1 \cdot \{CC_{BL_1}\}_{max}$, 7.07×10^{-4} s^{-1}) were calculated using Eq. (12). Compared to the values of the affinity constants for calcium ($\log K_{CaBL_1} = 3.47$), magnesium ($\log K_{MgBL_1} = 3.87$)and proton ($\log K_{HBL_1} = 5.13$), the value for sodium ($\log K_{NaBL_1} = 0.01$) indicates that copper bioaccumulation on bryophytes is not affected by Na concentrations in the exposure medium. The affinity constants value obtained for proton ($\log K_{HBL_1} = 5.13$), corresponding to the average p*K*a value of bryophyte binding sites, confirms the assumption of a total deprotonation at neutral pH, under which the H/Cu competition occurs. The stability constants ($\log K_{C_iBL_1}$) for Ca, Mg, and H obtained for *F. antipyretica* are on the same order of magnitude as those reported for fish gills (Santore et al., 2001; Niyogi et al., 2004) or daphnids (De Schamphelaere and Janssen, 2002).

4.4. Incorporation of cationic composition of water in the biomonitoring strategy

In order to illustrate how the modelling approach described in this paper could be incorporated in the (re)interpretation of

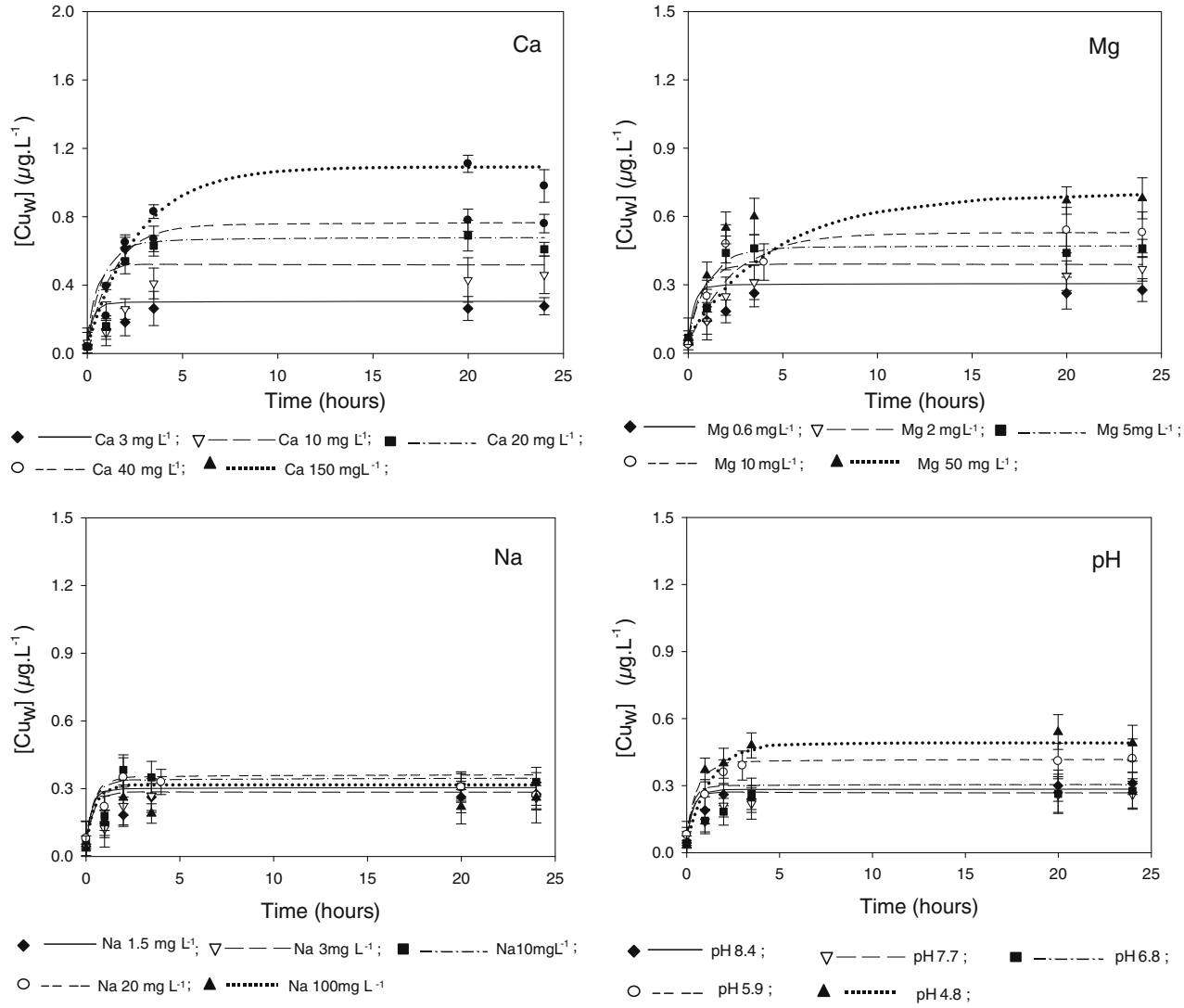

Fig. 3. Desorption kinetics of observed and calculated copper concentrations ([Cu_w]) during the 24-h desorption period.

bryophytes contamination levels commonly collected in monitoring networks, we selected two prospective situations. The first one deals with the interpretation of contamination levels of transplanted mosses, while the second one concerns contamination levels autochthonous bryophytes.

(i) Example 1: Influence of water cationic compositions for predicting copper bioaccumulation by transplanted mosses

In this example we evaluate the performance of the cationic-dependant kinetic model to describe the bioaccumulation in mosses transplanted in three contrasted water cationic compositions (representative of three French rivers). In this example, we suppose that bryophytes originating from a non-polluted area are transplanted on three different French rivers (the Loire, the Seine and the Moselle rivers, respectively). Average water cationic compositions of these rivers are reported in Table 2 (average values were determined from data collected by our institute during several in situ campaigns). In this predictive exercise, we considered that each of the selected rivers was submitted to an accidental contamination episode (96 h contamination phase with about 5 $\mu g L^{-1}$ of bioavailable Cu in water ([Cu_W]), Table 2). Evolutions in the bioaccumulated concentrations of copper ({Cu_m}) for mosses exposed to these three water cationic compositions (Moselle, Seine and Loire) can be calculated using the previously developed cationic-dependant kinetic model.

At the end of the 96 h contamination period, a fourfold difference for bioaccumulated copper ($\{Cu_m\}_{t=96h}$, Table 2) can be observed between the hard-water (the case of Moselle river) and soft-water (Loire case) scenarios. As regard to water quality classes (Table 2) used e.g. by French water management agencies to interpret bryophytes measurements, different 'pollution classes' would be drawn from bryophytes levels obtained on the Seine, Loire and Moselle rivers respectively (while the accidental scenario is considered to be the same one): the bioaccumulated copper obtained in the Loire river would correspond to the so-called 'Heavy pollution' class, while those observed at the Moselle river would correspond to the 'Safety range' class. Using the present cationic-dependant kinetic model, it is now feasible to predict the bioaccumulation of copper in aquatic mosses across a wide range of water quality conditions, and thus to derive site-specific WQC. For example, the use of the present cationic-dependant model may greatly improve the ability of regulators to (re)defined lower discharges thresholds for the Loire river (as compared to the Seine and Moselle rivers) in order to reach the recommended 'Safety Range' class.

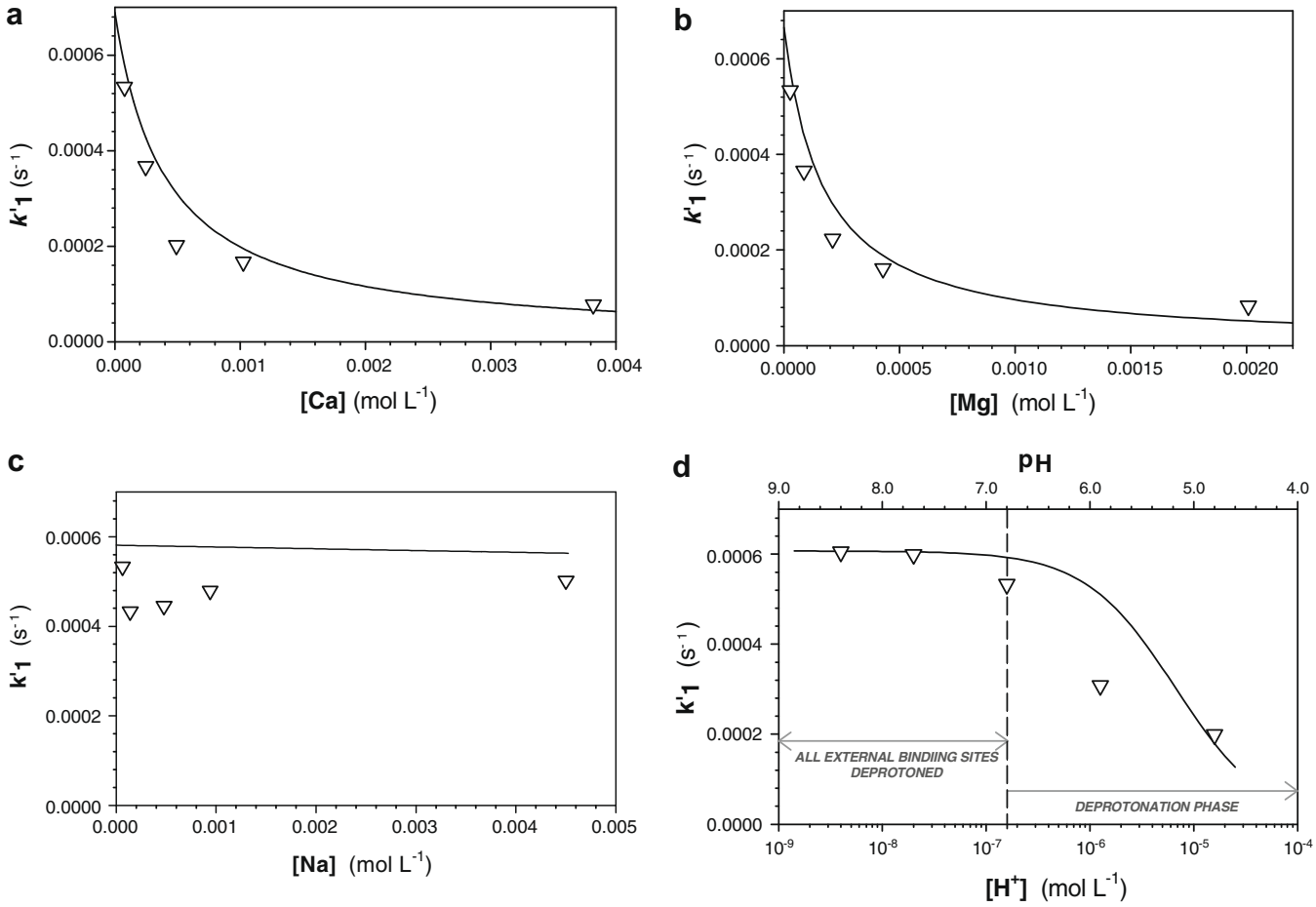

Fig. 4. Measured (▽) and calculated ((**––**); Eq. (12)) adsorption rate constant of copper (k'_1) by bryophytes as a function of the (a) Ca, (b) Mg, (c) Na concentrations, and (d) pH.

Table 2
Predicted kinetic constants and 96 h accumulated copper in mosses for three combinations of water cationic compositions (Loire, Seine and Moselle rivers cases) compared with French Water Criterion (WQC).

River	Input data					Kinetic parameters				Output data	Interpretation
	$[Cu_W]$ ($\mu g L^{-1}$)	Ca ($mg L^{-1}$)	Mg ($mg L^{-1}$)	Na ($mg L^{-1}$)	pH	[a] k'_1 (h^{-1})	k_{-1} (h^{-1})	k_2 (h^{-1})	k_{-2} (h^{-1})	$\{Cu_m\}_{t=96h}$ ($\mu g g^{-1}$ d.w.)	French Water Quality Criterion (WQC)
Loire	5	36	7	18	8.5	0.30	0.0576	0.0864	0.2952	239	$200 < \{Cu_m\}_{t=96h} < 400\ \mu g g^{-1}$ d.w. Heavy pollution
Seine	5	136	6	7	8.3	0.15	0.0576	0.0864	0.2952	123	$66 < \{Cu_m\}_{t=96h} < 200\ \mu g g^{-1}$ d.w. Proven pollution
Moselle	5	250	23	198	8.0	0.07	0.0576	0.0864	0.2952	54	$33 < \{Cu_m\}_{t=96h} < 66\ \mu g g^{-1}$ d.w. Safety range

[a] Conditional uptake rate constant calculated using Eq. (12).

(ii) Example 2: Evaluation of the bioavailable copper in water from measurements on autochthonous mosses

In this example, we investigate the use of the present model to quantify the theoretical bioavailable copper concentrations in water that would lead to a common aquatic mosses contamination in French river waters with a wide range of water cationic compositions. We assume that autochthonous mosses are at equilibrium in chronically Cu-contaminated rivers "*i*". For each river "*i*", characterized by its own cationic concentrations (Ca_i, Mg_i, Na_i, pH_i), Eqs. (1)–(3) can be simplified using the equilibrium assumption to calculate the bioavailable copper concentration in water $[Cu_W]_i$:

$$[Cu_W]_i = \frac{k_{-1,i}}{k'_{1,i}} \times \frac{\{Cu_m\}_i}{1 + \frac{k'_{2,i}}{k_{-2,i}}} \quad (13)$$

In this schematic exercise, we considered that bryophytes were collected in 80 different rivers ($i = 1$–80) and that copper bioaccumulated concentration is $100\ \mu g g^{-1}$ for each of them. Average cationic composition for these 80 rivers were obtained from national water agencies databases (data available at http://www.lesagencesdeleau.fr). For each of 80 water cationic composition (Ca_i varying from 1.3 to 165 $mg L^{-1}$, Mg_i from 0.6 to 38 $mg L^{-1}$, Na_i from 2.7 to 109 $mg L^{-1}$ and pH_i from 6.8 to 9.6), we calculated (Eq. (12)) the corresponding adsorption rate constant $k'_{1,i}$ (varying from

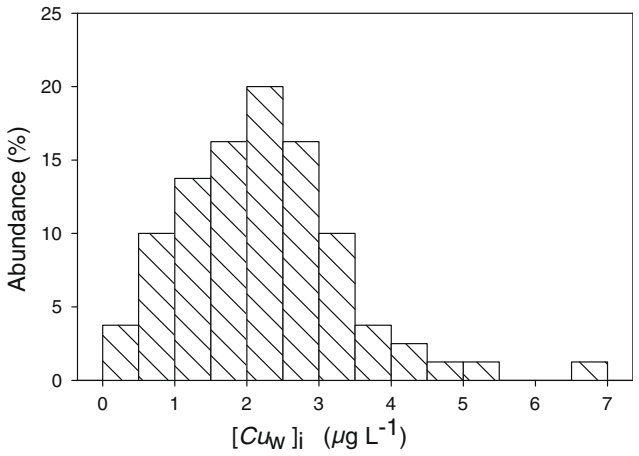

Fig. 5. Dispersion of the calculated bioavailable copper fraction ($[Cu_w]_i$) for the 80 rivers "*i*".

2.5×10^{-5} to 3.8×10^{-4} l g^{-1} s^{-1}). Each bioavailable copper $[Cu_W]_i$ was calculated using the previously estimated site-specific adsorption rate constant $k'_{1,i}$. The dispersion of the calculated bioavailable concentrations in water ($[Cu_W]_i$ for each *f* the 80 rivers "*i*" is graphically represented in Fig. 5. This results showed a 15-fold difference among the 80 calculated bioavailable concentrations (calculated $[Cu_W]_i$ varying from 0.44 μg L^{-1} to 6.7 μg L^{-1}), while the bioaccumulated copper concentration in mosses were assumed to be the same one. These results suggests that cationic composition of water have significant implication in the interpretation of autochthonous aquatic mosses contamination levels. The use of the present cationic-dependant model may greatly help national water management agencies in the evaluation of site-specific bioavailable metal contaminations in rivers.

5. Conclusions

Our experimental and modelling results have shown that water cationic composition have to be taken into account for predicting the bioaccumulation of copper by aquatic mosses. Based on the relationships between each major cation concentration and the copper exchange kinetics on mosses, the binding constants ($K_{C_i BL}$) of each competing cations to the biological surfaces were derived. We illustrate how a cation-dependent modelling approach could improve the interpretation of bryophytes measurements routinely collected in biomonitoring programmes. The experimental observations and the associated model developed in this paper may have important implications for the protection and management of freshwater biota in terms of national water quality guidelines. Hence, in the framework of future national water quality guidelines revisions, a such flexible and mechanistic biomonitoring tool (integrating the protective effects of competing cations) may greatly improve the ability of regulators to derive site-specific Cu (metal) guidelines for protecting aquatic biota, while limiting the use of conservative assumptions.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2008.10.031.

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